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DATE: Monday, August 06, 2007

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☐	L13	L12 not l4 not l2	55
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☐	L11	L10 and (vegatative or cytoplasmic or cytoplasma or intracellular or signal or rrna or r-rna or l1)	445
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☐	L6	L5 and (heterologous or heter-ologous or foreign or recombinantly or recombinant or engineering or delivery or fusion or chimeric or chimera or (expression near system))	18806
☐	L5	($spore or sporu$ or spore$)ti,ab,clm.	46588
		DB=EPAB,JPAB,DWPI; PLUR=YES; OP=OR	
☐	L4	L3 and (bacillus or spore or sporulation or vegatative or l1)	8
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☐	L2	L1 and ($spore or spor$ or $spore$)	7
☐	L1	rrno or rrn-o or (rrn near o) or rrn0 or rrn-0 or (rrn near 0)	385

END OF SEARCH HISTORY

☐ 1. WO2006087576A1. 20 Feb 06. 24 Aug 06. LISTERIOLYSIN-CONTAINING BACILLUS SPORES AS ANTIGEN DELIVERY AGENTS. CUTTING, SIMON.

☐ 2. WO2005068493A1. 17 Jan 05. 28 Jul 05. ANTHRAX VACCINE IN THE FORM OF A SPORE. CUTTING, SIMON MICHAEL. C07K014/32; A61K039/07.

☐ 3. WO003074682A1. 07 Mar 03. 12 Sep 03. BACTERIAL SPORES. CUTTING, SIMON MICHAEL. C12N003/00; C12N015/03 C07K014/195 A61K035/74.

☐ 4. WO003074681A1. 07 Mar 03. 12 Sep 03. RECOMBINANT SPORES. CUTTING, SIMON MICHAEL. C12N003/00; C12N015/00 A61K039/00.

☐ 5. WO2006087576A. New non-pathogenic Bacillus spores comprising a polynucleotide sequence encoding a hemolysin, e.g. listeriolysin O, useful for treating, preventing, or ameliorating infection, autoimmune condition, allergy, or cancer. CUTTING, S. A61K039/39 C12N015/87.

☐ 6. WO2005068493A. New non-pathogenic spore comprising an antigenic fragment of anthrax protective antigen, useful as an anthrax vaccine or for manufacturing an anthrax vaccine. CUTTING, S M. A61K039/07 C07K014/32.

☐ 7. WO2003074682A. New genetically modified spores comprising at least one genetic construct encoding an antigen and a spore coat protein as a chimeric gene, useful in the treatment of inflammation, pain, a hormonal imbalance and/or an intestinal disorder. CUTTING, S M. A61K035/74 A61K039/00 A61K039/02 A61K039/08 A61K039/108 A61K048/00 A61P031/04 C07K014/195 C12N001/21 C12N003/00 C12N015/03 C12N015/09 C12N015/74.

☐ 8. WO2003074681A. New spore useful for treating pain and inflammation, is genetically modified with genetic code comprising at least one genetic construct encoding a therapeutically active compound and targeting sequence or vegetative cell protein. CUTTING, S M. A61K035/74 A61K038/00 A61K038/22 A61K038/43 A61K039/00 A61K039/02 A61K039/08 A61K048/00 A61P001/00 A61P005/00 A61P025/04 A61P029/00 A61P037/04 C12N001/21 C12N003/00 C12N015/00 C12N015/09.

L1: Entry 30 of 34 File: USPT Nov 5, 1996

DOCUMENT-IDENTIFIER: US 5571698 A
** See image for Certificate of Correction **
TITLE: Directed evolution of novel binding proteins

Detailed Description Text (431):
It is believed that the conditions for an outer surface transport signal in a bacterial cell or spore are not particularly stringent, i.e., a random polypeptide of appropriate length (preferably 30-100 amino acids) has a reasonable chance of providing such a signal. Thus, by constructing a chimeric gene comprising a segment encoding the IPBD linked to a segment of random or pseudorandom DNA (the potential OSTS), and placing this gene under control of a suitable promoter, there is a possibility that the chimeric protein so encoded will function as an OSP-IPBD.

Detailed Description Text (434):
When the genetic package is a spore, we can use the approach described above for attaching a IPBD to an E. coli cell, except that: a) a sporulation promoter is used, and b) no periplasmic signal sequence should be present.

Parasporal

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☐ 1. Document ID: US 20050089959 A1

L3: Entry 1 of 3 File: PGPB Apr 28, 2005

DOCUMENT-IDENTIFIER: US 20050089959 A1
TITLE: Novel Bacillus thuringiensis strain, crystal gene and crystal protein and uses thereof

Brief Description of Drawings Paragraph:
[0052] FIG. 1 illustrates in panel A) a phase-contrast micrograph of a lysed culture of Bacillus thuringiensis strain M15; in panel B, a transmission electron micrograph of Bacillus thuringiensis strain M15 containing a spore and a tightly bound parasporal inclusion;

Brief Description of Drawings Paragraph:
[0058] FIG. 7 shows a transmission electron micrograph of a B. thuringiensis Cry.sup.- B transformant expressing the cry31Aa2 gene. S: spore; P: parasporal inclusion; Magnification: 20,000.times.;

Detail Description Paragraph:
[0063] A Bacillus thuringiensis strain was isolated from dead two-spotted spider mites (Tetranychus urticae Koch; Arthropoda: Arachnida: Tetranychidae) and named M15. The mites, parasitic on apple leaves, were collected in an apple orchard located in Frelighsburgh, Quebec, Canada. They were homogenized in 3 ml of phosphate-buffered saline (PBS) (NaCl 8 g, KCl 0.2 g, Na2HPO4 1.44 g, KH2PO4 0.24 g I-1). The homogenized solution was incubated for 16 hr at room temp and heated at 78.degree. C. for 15 min. Afterwards, the homogenate was plated on 2YT agar medium (Bacto Tryptone 16 g, Bacto Yeast Extract 10 g, NaCl 5 g, Agar 18 g I-1), and incubated for 24 hr at 30.degree. C. All colonies with a morphology similar to B. thuringiensis were streaked on T3 agar medium (Bacto Tryptone 3 g, Bacto Tryptose 2 g, Bacto Yeast Extract 1.5 g, MnCl2 0.005 g, 0.05M Sodium phosphate, pH6.7, Agar 18 g I-1) and incubated at 30.degree. C. for 48 hr. The cultures were examined by phase-contrast microscopy (Carl Zeiss Canada Ltd., Toronto, Ontario, Canada) for the presence of spores and crystals. B. thuringiensis M15 was deposited on 29 January 2001 in the International Depository Authority of Health Canada in Winnipeg under the Budapest Treaty (Bureau of Microbiology, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, R3E 3R2) under accession no. IDAC010201-5.

Detail Description Paragraph:
[0066] The parasporal inclusion bodies produced by a sporulated culture of B. thuringiensis strain M15 appear roughly spherical when observed under phase-contrast microscopy (FIG. 1A) and are tightly coupled to the spores even in lysed cultures. Further analysis under the transmission electron microscope (TEM), however, reveals that the parasporal inclusion body has a polygonal shape (FIG. 1B). The TEM observation was conducted after the B. thuringiensis strain M15 was incubated for 5 days at 30.degree. C. in T3 medium and the samples ultra-thinly sectioned according to Beveridge et al. (1994). Arrows show the roughly spherical

parasporal inclusions tightly bound to the white ovoid spores. In this figure, "S" and "P" denote spore and parasporal inclusion, respectively. Magnification used is of 25,000.times..

Detail Description Paragraph:
[0068] The B. thuringiensis strain M15 was grown in T3 medium for 5 days at 30.degree. C. on a rotary shaker to allow crystal protein production. Spores and crystals were separated from each other in the tightly bound parasporal duplexes using an ultrasonic processor model VC130 (Sonics & Materials, Inc., Newtown, Conn., USA) and purified by sucrose density gradient centrifugation as described elsewhere (Thomas and Ellar, 1983). Twenty microliters of the crystal suspension were added to 3 volumes of gel loading buffer (4% SDS, 20% glycerol, 125 mM Tris-HCl, 10% 2-mercaptoethanol, pH 6.8) in a 1.5-ml microtube, incubated at 90.degree. C. for 7 min and centrifuged for 2 min to remove unsolubilized materials. Thirty microliters of the supernatant were loaded on top of 10% SDS-polyacrylamide gels. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli and Favre (1973).

Detail Description Paragraph:
[0091] The B. thuringiensis Cry-B transformant containing the B. thuringiensis M15 parasporal crystal protein gene was incubated in nutrient broth (Bacto Beef Extract 3 g, Bacto Peptone 5 g I-1) at 30.degree. C. for 3 days to allow expression of the toxin gene and crystal formation. The presence of parasporal inclusions was examined by phase-contrast microscopy. When observed under a phase-contrast microscope, the B. thuringiensis transformants expressing the cry31Aa2 gene contained, in addition to the spore, a roughly spherical inclusion, whereas no inclusions were found in the B. thuringiensis transformant harboring the non-recombinant shuttle vector pHPS9 alone (data not shown). Under the transmission electron microscope (TEM), however, the parasporal inclusion body has a nearly perfect hexagonal shape (FIG. 7). Both inclusions in the transformant, spore and crystal, are separated from each other as opposed to what is found in B. thuringiensis strain M15 where they are tightly bound to each other.

Detail Description Paragraph:
[0094] The spore-inclusion mixture was harvested from sporulated cultures and the inclusions were partially purified by a biphasic separation method described in Goodman (1967) using polyethylene glycol 6000 (Wako Pure Chemical, Osaka, Japan) and sodium dextran sulfate 500 (Sigma, St. Louis, Mo.). Inclusions were further purified by sucrose density gradient centrifugation as described in Saitoh et al., (1998a). The purified inclusions were stored at 20.degree. C. until use.

Detail Description Paragraph:
[0120] 10. Goodman, N. S., R. J. Gottfried, and M. H. Rogoff. 1967. Biphasic system for separation of spores and crystals of Bacillus thuringiensis. J. Bacteriol. 94:485

Detail Description Paragraph:
[0121] 11. Haima, P., Van Sinderen, D., Schotting, H., Bron, S., and Venema, G. (1990). Development of a .beta.-galactosidase .alpha.-complementation system for molecular cloning in Bacillus subtilis. Gene 86, 63-69.

☐ 2. Document ID: US 20020182690 A1

L3: Entry 2 of 3 File: PGPB Dec 5, 2002

DOCUMENT-IDENTIFIER: US 20020182690 A1
TITLE: POLYHYDROXYALKANOATE BIOSYNTHESIS ASSOCIATED PROTEINS AND CODING REGION IN BACILLUS MEGATERIUM

Summary of Invention Paragraph:
[0008] A nucleic acid fragment encoding proteins involved in polyhydroxyalkanoate biosynthesis was isolated from Bacillus megaterium. Nine nucleic acid sequences and their encoded amino acid sequences are disclosed. Sequences encoding PhaB and PhaC display not insignificant percent identity and similarity to known acetoacetyl-CoA reductase and polyhydroxyalkanoate synthase proteins, while sequences encoding PhaP, PhaQ, and PhaR do not display significant similarity to known sequences. YkoY is similar to known toxic anion resistance proteins; YkoZ is similar to known RNA polymerase sigma factors; YkrM is similar to known Na.sup.+-transporting ATP synthase proteins; and SspD matches the known B. megaterium spore specific DNA binding protein.

Detail Description Paragraph:
[0142] Primer extension products showed a single band from each reaction, indicating one transcript, while control reactions in which RNA was omitted showed no bands. The extension products run alongside sequencing reaction products obtained with the same primer (FIG. 2C), identified the 5' ends of the transcripts thus allowing the putative promoter sequences at approximately -10 and -35-bp for phaP, -Q and -R to be identified. The arrangement of genes in the pha cluster of Bacillus megaterium is unique among those already published and phaA is notably absent. The phaP, -Q, -R, -B and -C genes were shown to be in a 4,104-bp region, with phaP and -Q transcribed in one orientation, each from a separate promoter, while phaR, -B and -C were divergently transcribed from a promoter in front of phaR. The putative promoters responsible for transcription of phaQ and phaR, phaB and phaC show strong similarity to both Bacillus subtilis Sigma A type (34) and Escherichia coli, Sigma 70 type promoters (14), which can express constitutively. This is in keeping with previous data for Alcaligenes eutrophus showing that phaC is constitutively synthesized, but PHA is not constitutively accumulated (19). The third putative promoter in this region, the phaP promoter, resembles a Sigma D (SigD) type promoter known to control the expression of a regulon of genes associated with flagellar assembly, chemotaxis and motility (13, 20, 46). In Bacillus subtilis Sigma D is expressed in the exponential phase and peaks in late exponential phase of growth. This parallels the pattern of PHA accumulation previously described for Bacillus megaterium 11561 (32). However, further experiments are required to test the hypothesis that PHA accumulation in regulated by sigma D or products of its resulting transcripts. The phaP gene has 18-bp duplicate sequences that could base-pair to form a rho-independent terminator close to its translational stop codon (FIG. 2B). The fact that the -35 promoter region of sspD is within this putative hairpin structure, suggests that transcription of phaP and sspD could be mutually exclusive, thus allowing the expression of phaP to play a regulatory role in the expression of sspD (spore specific storage protein).

Detail Description Paragraph:
[0193] 4. Connors, M. J., J. M. Mason, and P. Setlow. 1986. Cloning and nucleotide sequencing of genes for three small, acid soluble proteins Bacillus subtilis spores. J. Bacteriol., 166: 417-425.

Detail Description Paragraph:
[0198] 9. Fliss, E. R., A. C. Loshon, and P. Setlow. 1986. Genes for Bacillus megaterium small, acid-soluble spore proteins: Cloning and nucleotide sequence of three additional genes from this multigene family. J. Bacteriol., 165: 467-473.

Detail Description Paragraph:
[0199] 10. Fliss, E. R. and P. Setlow. 1984. Bacillus megaterium spore protein C-3:

nucleotide sequence of its gene and the amino acid sequence at its spore cleavage site. Gene, 30: 167-172.

Detail Description Paragraph:
[0205] 16. Haima, P., D. van Sinderen, H. Scholting, S. Bron, and G. Venema. 1990. Development of .beta.-galactosidase .alpha.-complementation system for molecular cloning in Bacillus subtilis. Gene, 86: 63-69.

Detail Description Table CWU:
5TABLE 4 Sequence homologies Homologies to known and Sequence putative genes (accession no.).sup.a Identity Similarity Function or putative function ykoY YkoY, B. subtilis (Z99110) 64% 73% Toxic anion resistance protein (24) ykoZ YkoZ, B. subtilis (Z99111) 57% 74% RNA polymerase sigma factor (24) sspD SspD, Bacillus megaterium 100% Spore specific, DNA binding (P10572) protein (4, 10) SspD, B. subtilis (P04833) 73% 87% phaP None PHA inclusion-body structure, shape and size (49) phaQ None Unknown phaR None Unknown phaB FabG, Synechocystis (D90907) 50% 66% Fatty acid biosynthesis (23) PhaB, C. vinosum D (P45375) 48% 64% 3-ketoacyl-CoA reductase (28) FabG, B. subtilis (P51831) 47% 67% Fatty acid biosynthesis (35) phaC PhaC, T. violacea (P45366) 38% 59% PHA synthase (29, 23, 28) PhaC, Synechocystis (D90906) 37% 56% PhaC, C. vinosum (P45370) 35% 55% ykrM YkrM, B. subtilis (Z99111) 55% 71% Na.sup.+-transporting ATP synthase (24) .sup.aAccession numbers are SWISS-PROT, EMBL or DDBJ; .sup.bNone, No discernible similarity to known sequences.

☐ 3. Document ID: US 6835820 B2

L3: Entry 3 of 3 File: USPT Dec 28, 2004

DOCUMENT-IDENTIFIER: US 6835820 B2
TITLE: Polyhydroxyalkanoate biosynthesis associated proteins and coding region in bacillus megaterium

Brief Summary Text (10):
A nucleic acid fragment encoding proteins involved in polyhydroxyalkanoate biosynthesis was isolated from Bacillus megaterium. Nine nucleic acid sequences and their encoded amino acid sequences are disclosed. Sequences encoding PhaB and PhaC display not insignificant percent identity and similarity to known acetoacetyl-CoA reductase and polyhydroxyalkanoate synthase proteins, while sequences encoding PhaP, PhaQ, and PhaR do not display significant similarity to known sequences. YkoY is similar to known toxic anion resistance proteins; YkoZ is similar to known RNA polymerase sigma factors; YkrM is similar to known Na.sup.+ -transporting ATP synthase proteins; and SspD matches the known B. megaterium spore specific DNA binding protein.

Detailed Description Text (139):
Primer extension products showed a single band from each reaction, indicating one transcript, while control reactions in which RNA was omitted showed no bands. The extension products run alongside sequencing reaction products obtained with the same primer (FIG. 2C), identified the 5' ends of the transcripts thus allowing the putative promoter sequences at approximately -10 and -35-bp for phaP, -Q and -R to be identified. The arrangement of genes in the pha cluster of Bacillus megaterium is unique among those already published and phaA is notably absent. The phaP, -Q, -R, -B and -C genes were shown to be in a 4,104-bp region, with phaP and -Q transcribed in one orientation, each from a separate promoter, while phaR, -B and -

C were divergently transcribed from a promoter in front of phaR. The putative promoters responsible for transcription of phaQ and phaR, phaB and phaC show strong similarity to both Bacillus subtilis Sigma A type (34) and Escherichia coli, Sigma 70 type promoters (14), which can express constitutively. This is in keeping with previous data for Alcaligenes eutrophus showing that phaC is constitutively synthesized, but PHA is not constitutively accumulated (19). The third putative promoter in this region, the phaP promoter, resembles a Sigma D (SigD) type promoter known to control the expression of a regulon of genes associated with flagellar assembly, chemotaxis and motility (13, 20, 46). In Bacillus subtilis Sigma D is expressed in the exponential phase and peaks in late exponential phase of growth. This parallels the pattern of PHA accumulation previously described for Bacillus megaterium 11561 (32). However, further experiments are required to test the hypothesis that PHA accumulation in regulated by sigma D or products of its resulting transcripts. The phaP gene has 18-bp duplicate sequences that could base-pair to form a rho-independent terminator close to its translational stop codon (FIG. 2B). The fact that the -35 promoter region of sspD is within this putative hairpin structure, suggests that transcription of phaP and sspD could be mutually exclusive, thus allowing the expression of phaP to play a regulatory role in the expression of sspD (spore specific storage protein).

Detailed Description Text (204):

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Detailed Description Paragraph Table (5):
TABLE 4 Sequence homologies Homologies to known and Sequence putative genes (accession no.).sup.a Identity Similarity Function or putative function ykoY YkoY, B. subtilis (Z99110) 64% 73% Toxic anion resistance protein (24) ykoZ YkoZ, B. subtilis (Z99111) 57% 74% RNA polymerase sigma factor (24) sspD SspD, Bacillus megaterium 100% Spore specific, DNA binding (P10572) protein (4, 10) SspD, B. subtilis (P04833) 73% 87% phaP None PHA inclusion-body structure, shape and size (49) phaQ None Unknown phaR None Unknown phaB FabG, Synechocystis (D90907) 50% 66% Fatty acid biosynthesis (23) PhaB, C. vinosum D (P45375) 48% 64% 3-ketoacyl-CoA reductase (28) FabG, B. subtilis (P51831) 47% 67% Fatty acid biosynthesis (35) phaC PhaC, T. violacea (P45366) 38% 59% PHA synthase (29, 23, 28) PhaC, Synechocystis (D90906) 37% 56% PhaC, C. vinosum (P45370) 35% 55% ykrM YkrM, B. subtilis (Z99111) 55% 71% Na.sup.+ -transporting ATP synthase (24) .sup.a Accession numbers are SWISS-PROT, EMBL or DDBJ; .sup.b None, No discernible similarity to known sequences.

Term	Documents
SPORE	18401
SPORES	29862
(2 AND SPORE).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	3
(L2 AND SPORE).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	3

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/000582

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/39 C12N15/87

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12R C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
1 June 2006	22/06/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van de Kamp, M

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/000582

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International application No
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Information on patent family members

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